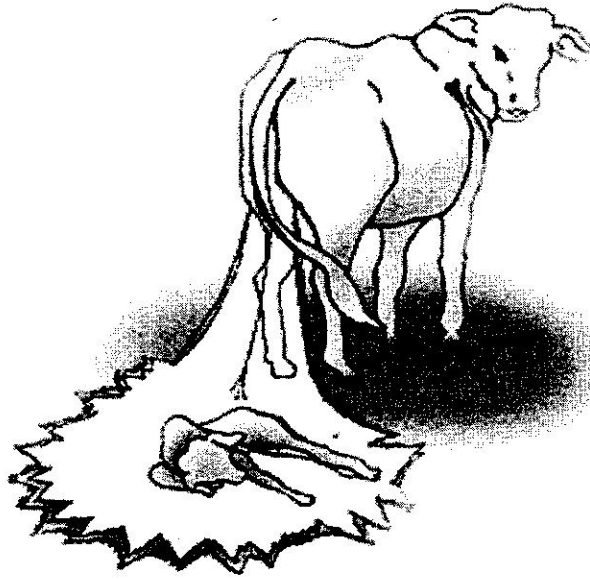


Gram -Ve Bacteria

(1)

Genus: Brucella

(مرض الاجهاض المعدي في الأبقار)



أ.د/ جمال يونس

(3) Phylum(Division):

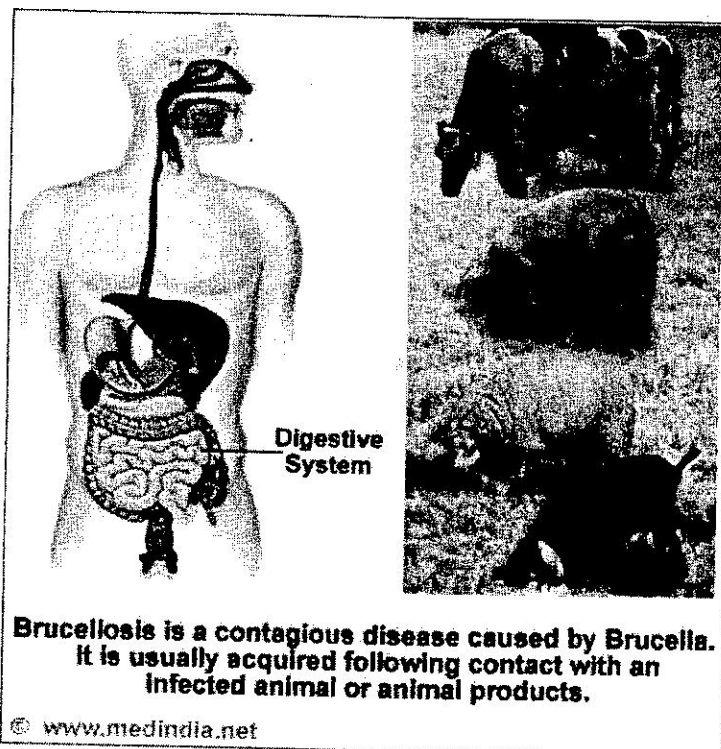
Proteobacteria

Class: α - Proteobacteria

Order: Rhizobiales

Family: brucellaceae

Genus: **Brucella**



Genus: Brucella

Species :

- ① *B. abortus* → Cattle
② *B. melitensis* → sheep and goats
③ *B. suis* → pigs
④ *B. neotomae* → desert wood rat
⑤ *B. ovis* → sheep
⑥ *B. canis* → dogs
- The 3 principal (main) brucella species

- ◆ The first 4 species are Smooth strains.
The Last 2 species are Rough strains.

• Smoothness and Roughness of brucella →
depend on the presence of surface antigens
(A, M and R) (Lps) on the cell wall of
brucella → A and M antigens (smooth strains)
→ R antigen (Rough strains)

pathogenicity:

- Brucellae are obligate pathogens and facultative intracellular parasites → Where they are phagocytosed and remain viable inside phagocytes through the prevention of fusion of lysosomes with phagosomes.

◆ Cause brucellosis (Contagious abortion, Bang's disease) in ♀ cattle, sheep and goats
→ ch' by:

- 1- Storm of abortion (80-90%) esp. at the Late stage of pregnancy.
- 2- Retention of placenta

◆ ♂ animals:

- 1- Infertility
- 2- epididymitis, orchitis, testicular abscess
- 3- hygroma of joints (arthritis)
- 4- B. ovis → Cause Ram epididymitis.

◆ Human:

It is a zoonotic disease.

- 1- Cause → Malta fever (B. melitensis)
 undulant fever (B. abortus, B. suis, B. canis)
- 2- Man can be infected by all brucella species except B. ovis and B. neotomae.
- 3- No abortion or osteomyelitis in man.
- 4- Human in close contact with infected animals (slaughterhouse workers, vets, farmers and dairy workers) are at risk of developing undulant fever (occupational disease)

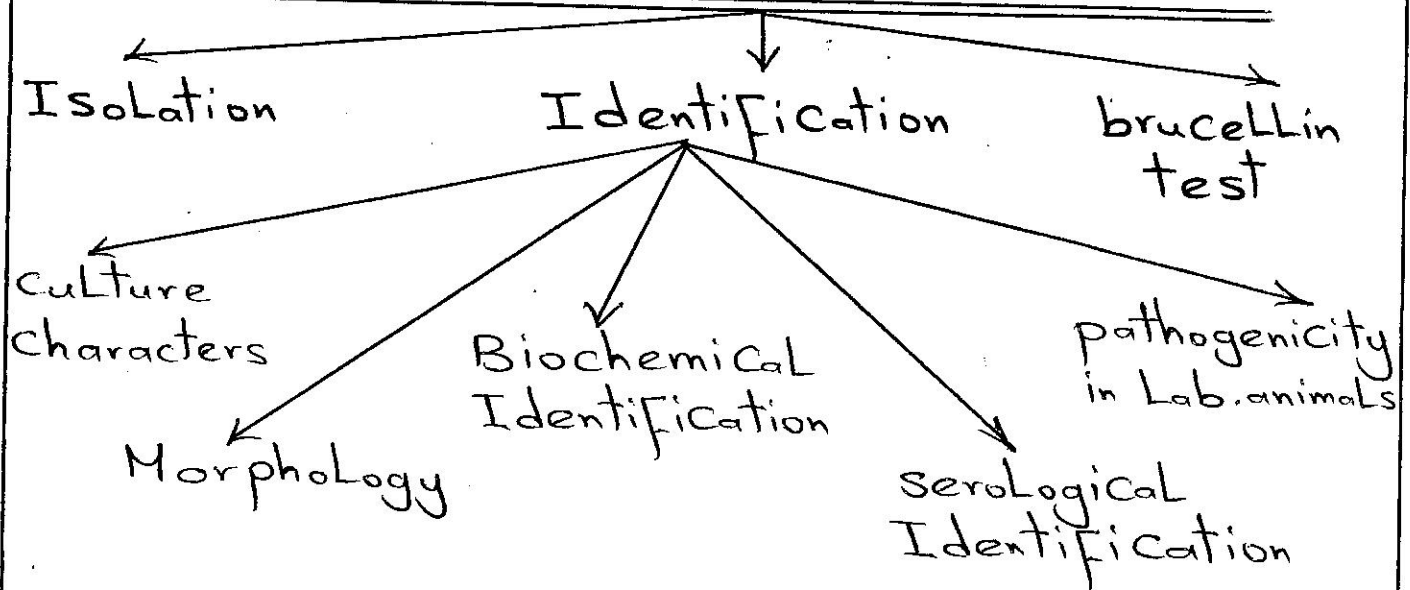
- The predilection sites of brucellae are the reproductive tracts of ♂ and ♀ esp. the pregnant uterus → because erythritol (which is essential for the growth of most brucellae) is present in the placenta and ♂ genital tract of cattle, sheep, goats and pigs except humans.
- Females usually abort only once, after which a degree of immunity develops and the animals become carrier → excrete large No. of brucellae in fetal fluids at subsequent parturitions.

Laboratory diagnosis :

Specimens :

- 1- Fetal membranes
- 2- Fetal stomach contents
- 3- Internal organs (Liver and spleen) and LNs
- 4- uterine exudate of recently aborted animal.
- 5- semen, testicles and preputial washings
- 6- abscesses and other ext. lesions (e.g. hygroma of joints)
- 7- MILK
- 8- serum of suspected animals for serological tests.

Laboratory diagnosis of brucella :



(A) Isolation :

- O₂ requirement → aerobic except B. abortus requires 5-10% CO₂ to grow (Capnophilic)
- opt. temp. → 35-37 °C
- pH of media → slightly acidic (pH 6.6-6.8)
- Incubation time → 48 hrs - 4 weeks
- Brucellae grow slowly on ordinary media (fastidious organism) → Their growth is improved by the addition of blood, serum or tissue extracts.
- Brucellae are unable to grow on MacConkey's agar.

- ◆ The general (basal) media used for the isolation of brucellae are :

↓ Liquid media	Solid media ↓
① Serum dextrose tryptone soya broth (SDB) ② Albimi brucella broth (ABB)	① Serum dextrose agar (SDA) ② Liver infusion agar ③ Albimi brucella agar (ABA)

- ◆ The basal media become selective for the isolation of Brucella by addition of :

- ① antibiotics and antifungal agents → such as polymyxin B, bacitracin, actidione and Circulin.
- ② dyes → such as thionin, basic fuchsin, crystal violet and pyronin.
→ to inhibit the growth of other M.O

⑧ Identification :

1. Culture characters :

↓ Smooth Colonies	↓ Rough Colonies
small, convex, round, grayish and translucent which become opaque and brownish with age	dull, opaque, yellowish and granular with irregular border.
◆ No hemolysis on blood agar.	

2. Morphology:

◆ stain used :

- Gram's stain
- Modified Ziehl-Neelsen's stain
- specific stains → such as Kazlovski stain or Koster's stain.

◆ staining reaction :

1. Gram's stain → Gram -ve
2. Modified Z.N → Brucellae are non-acid fast organisms but they resist the decolorization by weak acids (such as 0.5 % acetic acid) → So, they are stained red and other organisms are stained blue → So, they are weak acid fast organisms.

Q : Why Brucella is weak acid fast organisms ?

because they resist decolorization by weak acids (such as 0.5 % acetic acid) → So, they are stained red and other organisms are stained blue by modified Ziehl-Neelsen's stain.

◆ Shape : Coccobacilli (pleomorphic)

◆ Size : small

◆ arrangement : single, pairs or in short chains.

◆ Non-sporulated

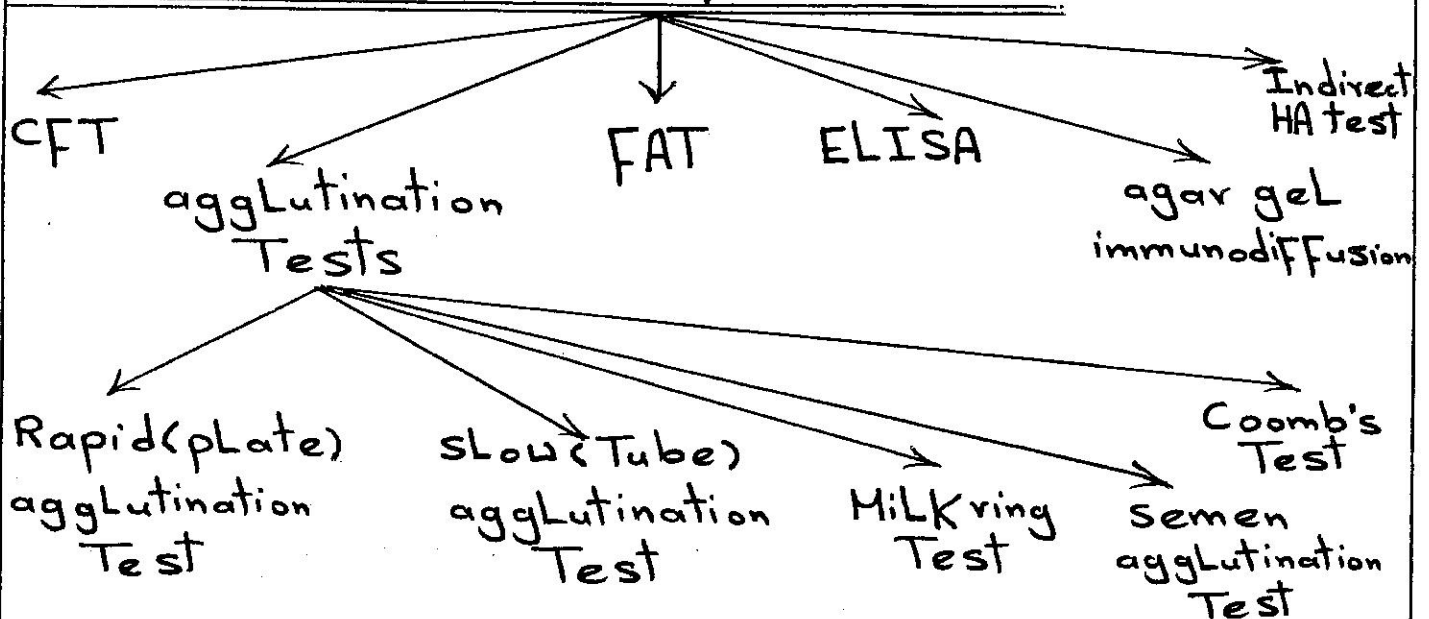
◆ Non-capsulated

◆ Non-motile

3. Biochemical identification:

- ◆ Catalase +ve except *B. ovis* and *B. neotomae*
- ◆ Oxidase +ve except *B. ovis* and *B. neotomae*
- ◆ Less active in Carbohydrate fermentation.

4. Serological identification:



① Complement fixation test (CFT):

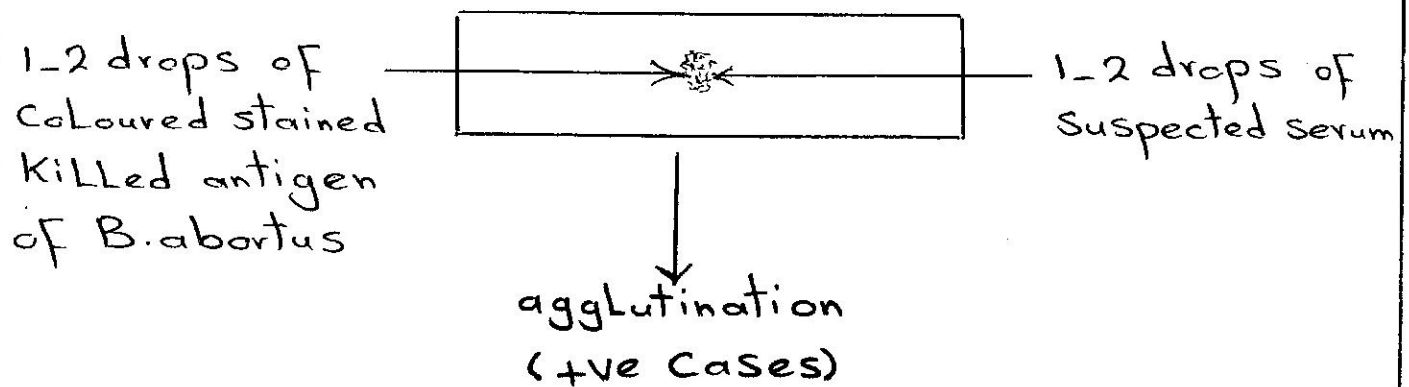
- ◆ It is highly specific and very sensitive depending on IgM and IgG₁
- ◆ Complement fixing antibodies appear in the blood of infected animals shortly before agglutinins.

② Agglutination tests :

a- Rapid (plate) agglutination test:

1- Coloured stained killed antigen of B. abortus:

used for control of disease and depends on IgM and IgG₁



2-Rose-Bengal plate test:

- ♦ antigens → buffered to pH 3.65 - 4
→ stained with Rose-Bengal stain (Red)
- ♦ Serum → diluted with acid buffer (pH 3.5) to destroy IgM (non-specific antibodies) Leaving IgG (specific antibodies) → so, this test is more accurate.

3- Rivanol test:

depends on precipitation of IgM (mostly produced by adult vaccination) by treating the serum with RiVanol solution.

b. SLOW (Tube) agglutination test :

1. ordinary tube agglutination test :

- It is a standard test used for animals positive for plate agglutination test.
- used routinely in the farms.
- depends on IgM and IgG₂
- done by making 2-fold dilutions of serum

Result:

	Cattle	sheep	Human
suspicious	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{80}$
positive	$\frac{1}{40}$	$\frac{1}{80}$	$\frac{1}{160}$

→ suspicious cases are re-tested after 10-15 days because agglutinins appear in the blood 10 days post-abortion.

2. Mercaptoethanol test :

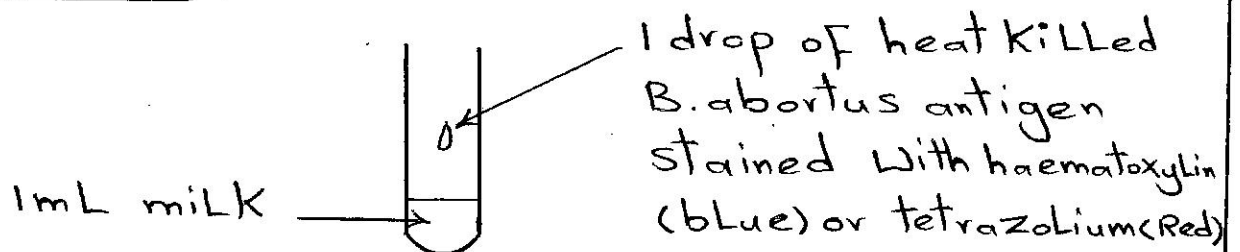
In which, IgM antibodies are destroyed
(These antibodies are mostly produced by adult vaccination)

c-Milk ring test

(Abortus Bang Ring test, ABR)

- depends on the presence of IgM, IgG, and IgA
- This test depends on that the agglutinins which are excreted in milk during infection are carried to the surface with the rising fat globules.

procedure:



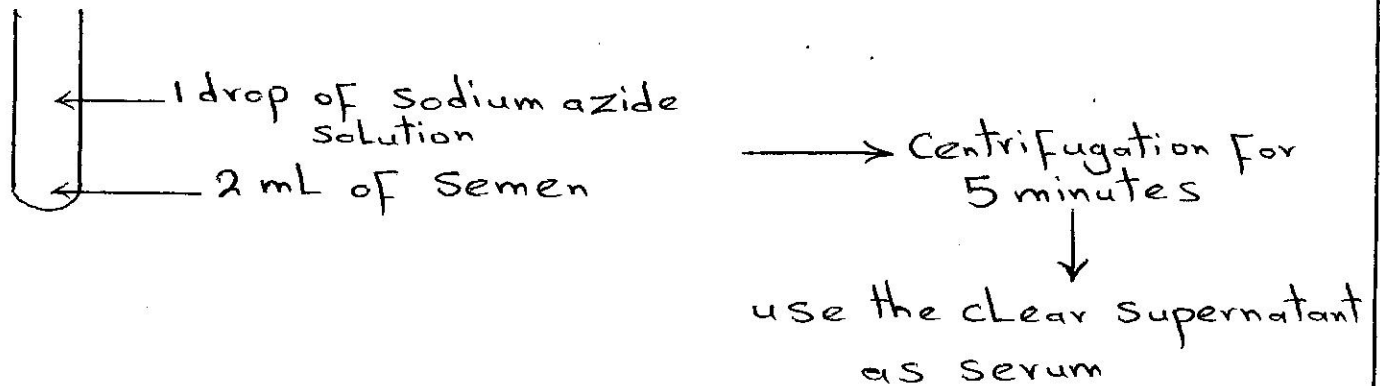
Incubate in water bath at 37°C for 1 hr

Result:

Cream Layer	Coloured	unColoured	Coloured
Milk	unColoured	Coloured	Coloured
Result	+ve	-ve	suspicious

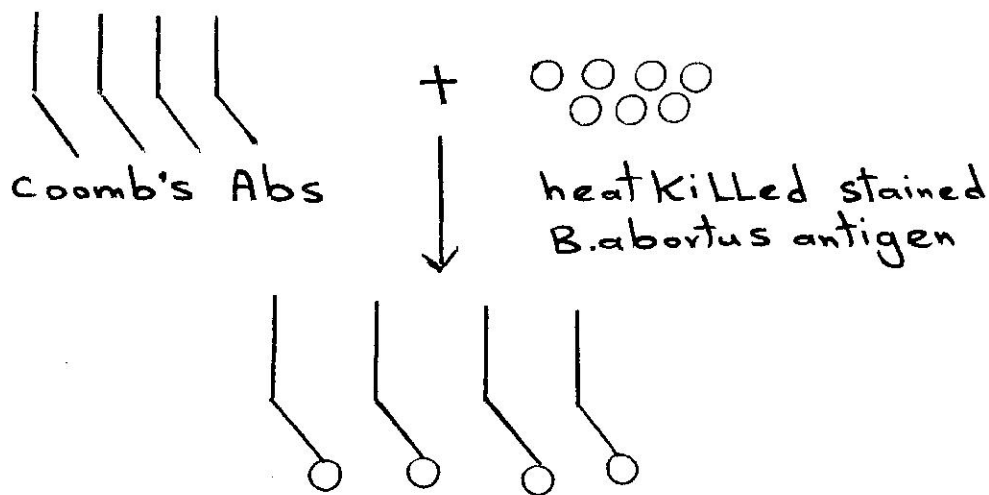
→ suspicious Cases are retested after 10-15 days

d - Semen agglutination test :

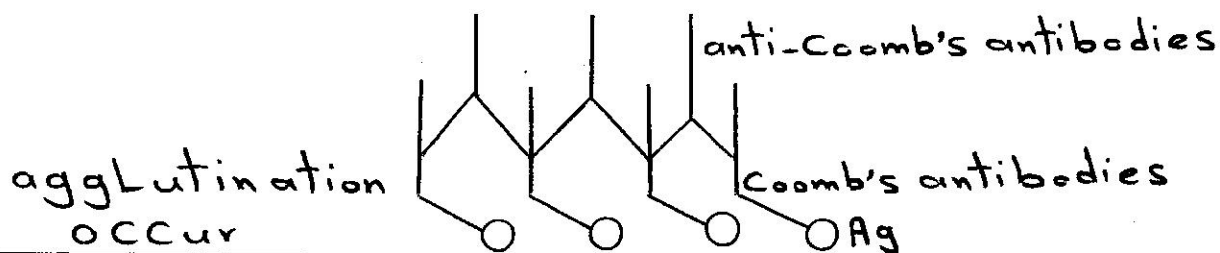


e - Coomb's test :

Very sensitive in chronic infection → due to the production of Coomb's antibodies (Incomplete antibodies contain one Fab portion only)

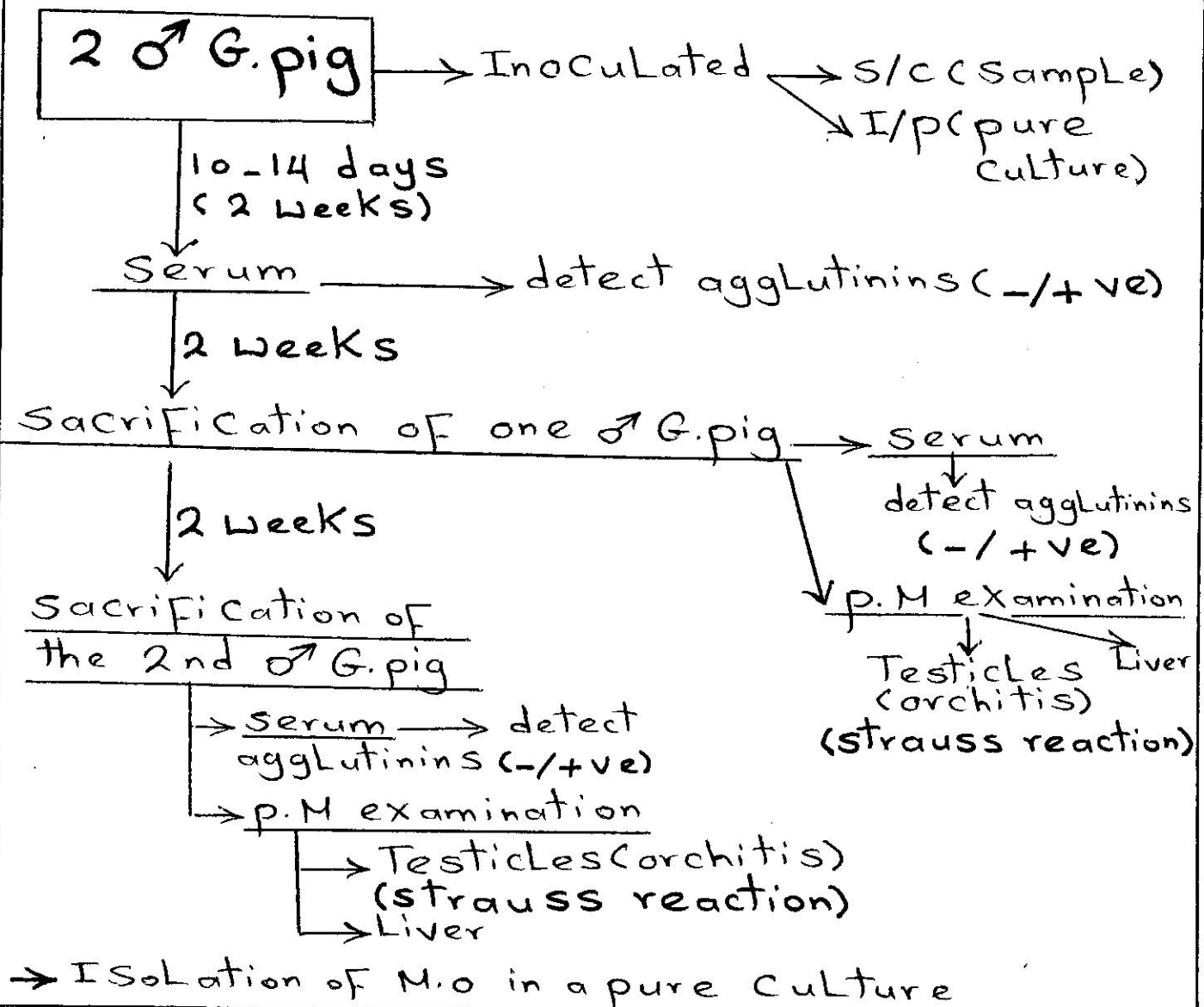


So, use anti-Coomb's antibodies (antiGlobulins)



5-pathogenicity in Lab. animals:

- It is used for diagnosis as well as for getting a pure culture of the organism.
- Blood samples should be taken from ♂ G. pig before inoculation and tested for the presence of agglutinins.



N.B.:

- The sample is inoculated s/c because it is very highly contaminated → where it is filtrated in Ln → followed by M.o proliferation
- Bacteria Causing strauss reaction:
 - Brucella
 - Burkholderia mallei
 - Corynebacterium ovis (C. ovis)

© Brucellin test:

It is allergic skin test depends on delayed type of hypersensitivity.

- In suspicious cases to be infected with brucella, I/D injection of brucellin (specific protein extract of the organism) → result in activation of sensitized T-Lymphocytes to secrete lymphokines (cytokines) which are responsible for skin reaction.
 - 1- chemotactic factor → attraction of macrophage and uncommitted (unsensitized) lymphocytes to site of injection.
 - 2- MIF → inhibit migration of macrophage

3- γ -IFN \rightarrow activate macrophage

4- Macrophage aggregation Factor (MAF)

\rightarrow giant cell formation (chronic disease)

5- skin permeability factor \rightarrow \uparrow capillary permeability

+ve reaction:

oedema then Induration (measured by Caliper)

- It is satisfactory for diagnosis of Malta fever in human but unreliable in Cattle.

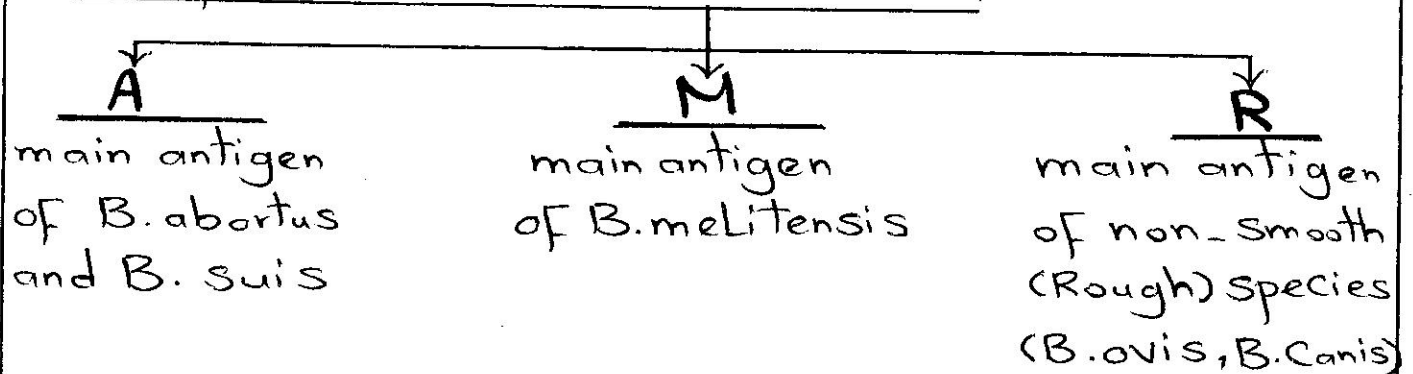
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01003912810

differentiation of brucella species

a. serotyping of brucella:

→ there are 2 surface antigenic determinants (A and M) shared among the 3 principal brucella species → So, they are responsible for the cross reactions (i.e. antisera from immunized animals with one smooth strain will agglutinate the 3 principal brucella species)

→ these surface antigens of brucella are determined by the composition of LPS Complexes in the cell wall.



→ antigenic A : M ratio :

3 principal brucella spp.	A	M
1- <i>B. abortus</i> and <i>B. suis</i>	20	1
2- <i>B. melitensis</i>	1	17.5

→ Serotyping of brucella is carried out either by slide or tube agglutination test using monospecific A, M and R antisera.

→ Common Ag of brucellae (LPS antigen) cross react with E. coli, salmonellae, pseudomonas spp., campylobacter spp. and vibrio cholerae.

b. differential characteristics of the three principal brucella species:

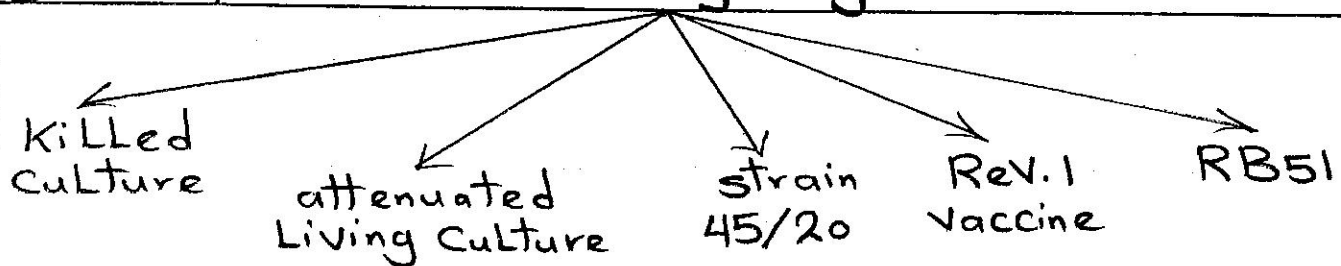
Points of differences	B. abortus	B. melitensis	B. suis
1- CO ₂ requirement	+	-	-
2- H ₂ S production	+	-	+
	(after 2 days)		(after 4 days)
3- Hydrolysis of urea	Slow (1-2 hrs)	Variable reaction	Rapid (within 1/2 hr)
4- growth on dye containing media:			
● Thionin 1/50,000	-	+	+
● Methyl violet 1/50,000	+	+	-
● Basic Fuchsin 1/50,000	(+)	+	(-)
● pyronin 1/100,000	+	+	-
5- agglutination using monospecific antisera:			
● A (abortus)	(+)	(-)	(+)
● M (melitensis)	(-)	(+)	(-)

Immunity (Immunization or Vaccination against brucella)

a) Natural Immunity:

- young animals are more resistant to infection than adult ones due to presence of maternal immunity.
- Recovered animals develop state of immunity but still excrete the organism in their discharge.

b) artificial immunity by vaccination:



1. Killed Culture (Bacterin):

Killed Culture of *B. abortus* either by heat or chemicals with oil adjuvant.

- It is not harmful and animals are vaccinated twice by it.
- produce neither solid nor lasting immunity because it produce humeral immunity but not cell mediated immunity (which is protective)
- produce Complement Fixing antibodies.

2- attenuated Living Culture:

→ B. abortus strain 19 (Book strain) →
used for immunization of cattle at age
of 6-8 months old → S/C (single injection)

● animals ↓ 5 months old should not be vaccinated:

- 1- to avoid the interference with maternal immunity.
- 2- cells and tissues of the immune system will not be so highly developed to form a level of Abs sufficient to produce good protection.

● disadvantages:

- ① adult animals develop permanent agglutinins
which interfere with agglutination test results
→ so, it is difficult to differentiate between the diseased one and the vaccinated one.
- ② may cause severe postvaccination reaction
→ abortion in pregnant cattle.
- ③ Contraindicated in vaccination of ♂ at any age
 - because it produce permanent agglutinin and so can't differentiate between the infected male and vaccinated one.
 - may cause infertility in some ♂ calves

3. strain 45/20 :

It is rough strain of *B. abortus*.

→ the strain No. 45 is subcultured 20 times.
disadv. :

Live rough strain of *B. abortus* 45/20 is virulent in vivo.

4. Rev. 1 vaccine :

→ prepared from *B. melitensis*

→ used for vaccination of sheep and goats

→ given S/C

5. RB 51 :

It is rough strain of *B. abortus*

Q: Which is better for vaccination against brucella, killed culture or strain 19?

1. strain 19 is more preferred → because it produces cell mediated immunity which is protective against brucella.

2. While killed culture produces humeral immunity which is not protective.

• Immunity in brucellosis is predominantly cell-mediated.